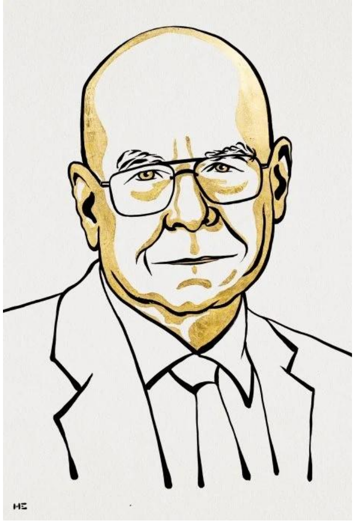
NOBEL PRIZES IN SCIENCE 2022
AN APPRECIATION

S T Lakshmikumar

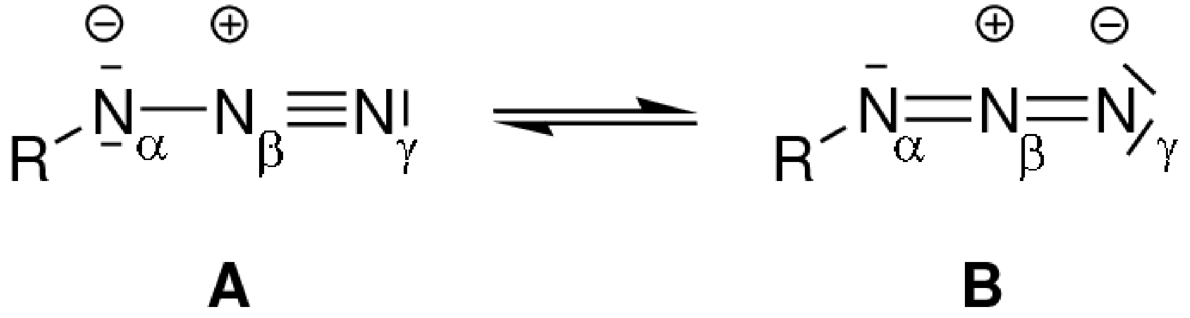
This is an attempt to appreciate the science which has been recognized and rewarded with Nobel prizes in Chemistry, Physics and Medicine for the year 2022, an annual effort on my part for the past many years.



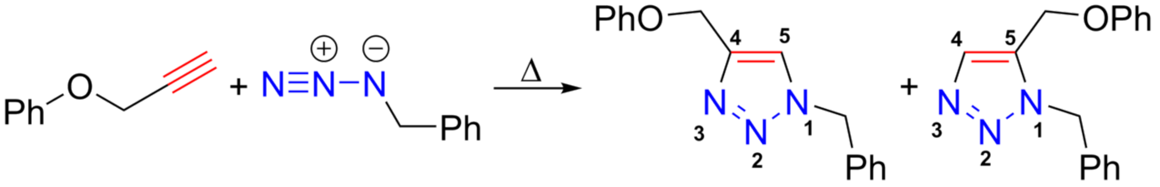
Carolyn R. Bertozzi. Morten Meldal K. Barry Sharpless

The Nobel Prize in Chemistry 2022 was awarded jointly to Carolyn R. Bertozzi, Morten Meldal and K. Barry Sharpless "for the development of click chemistry and bioorthogonal chemistry"

"Click Chemistry", a term coined by Sharpless in 2001 and "Bioorthogonal Chemistry", the term coined by Bertozzi in 2002 are very obvious ideas. Let us consider click chemistry first. Any molecule "AB" consists of a number of atoms bonded together in a particular way. A and B themselves are not individual atoms except in the simplest case. A chemist tries to produce AB. But A and B are not usually freely available. So AB is produced by the reaction between AC and BD which results in the desired product AB and also the unwanted residues like C', D', CD etc. It is trivially obvious that a reaction which proceeds rapidly to completion at room or low temperature and produces benign residues like water or oxygen thereby enabling easy separation of the desired product AB would be very useful. Unfortunately such reactions would be very rare. Sharpless visualised click chemistry as an approach of first identifying two small molecules x and y which have these desirable reaction characteristics and can also be individually attached to a wide range of A's and B's. Then the reaction of any combination of one of the A's and one of the B's simplifies to the reaction of Ax and By. The addition of the small molecular link xy {AxyB} would not matter if A and B were much larger entities than x and y. Sharpless was clearly influenced by the peptide bond between the amino acids in proteins and the phosphodiester bond between nucleotides in DNA. Sharpless identified several potential candidates for click reactions, so called because the extremely fast xy bond formation would make the bonding of AB resemble snapping together of building blocks. One of the reactions he identified as a potential candidate is the 1-3 dipolar cycloaddition of an azide and an unsaturated hydrocarbon, an alkene or an alkyne.



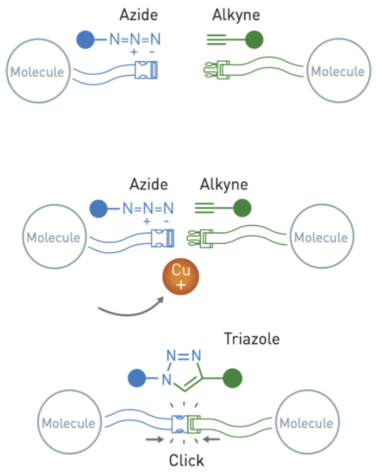
Formation of organic azides RN_3 and their chemical properties were reported as early as 1864. In 1933, Linus Pauling described the dipolar resonance structures of Azides which shows in the figure, the dipole on adjacent nitrogen atoms while Adler reported the reaction between phenyl azide and an alkene.



1 **2** **3a** **3b**

Huisgen in 1954 proved that the high reaction rate of the cycloaddition was due to simultaneous bond formation leading to the five membered heteroatomic ring with three nitrogen atoms rather than a slower two step process. Nitrogen atoms 1,3 in the figure bond simultaneously with the alkyne. So the fundamental understanding of the cycloaddition already existed and was doubtless responsible for Sharpless identifying its potential use in click chemistry.

The next step in click chemistry was the identification of copper as a catalyst for the azide alkyne reaction by Meldal. It was a serendipitous result and initially, Meldal seems not to have realised the importance of the result. He had attached the alkyne to a solid suport and used Cu^{1+} as the catalyst. Simultaneously and apparently independently, Sharpless demonstrated the use of Cu^{2+} salts in liquid medium as a catalyst and identified it as an ideal click reaction. The use of copper as a catalyst caused a million fold increase in the reaction rate and it was insensitive to the molecules attached to the azide and terminal alkyne. This made it the textbook example of a click reaction. x and y in the description above are the azide and terminal alkyne groups and Cu catalyses the reaction for any A and B attached.



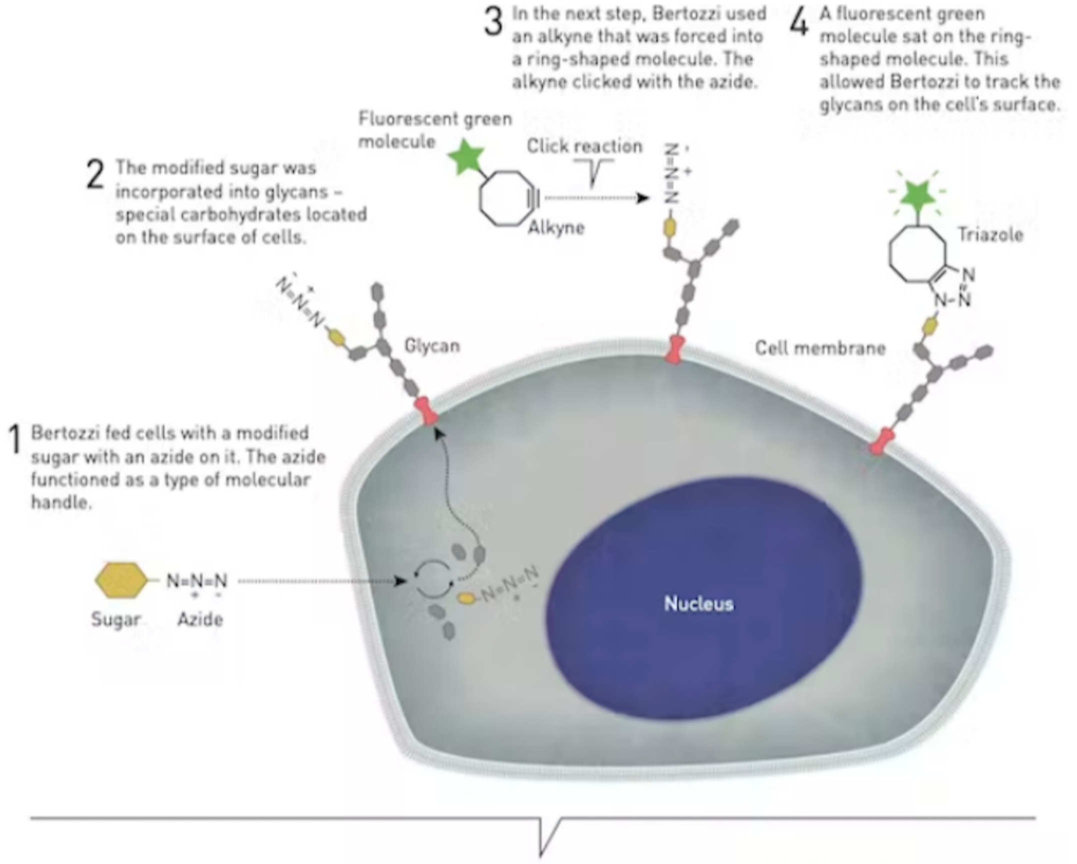
It is a remarkable coincidence that this reaction, which is still the best example of a click reaction, was discovered just one year after Sharpless proposed click chemistry. The triazole (5 member ring with 3 nitrogen atoms) is extremely inert. It does not for example undergo oxidation, reduction or hydrolysis.

The discovery of copper catalysed azide alkyl cycloaddition CuAAC and its improvements using Palladium or Rhenium has been revolutionary. It has been employed in diverse areas such as functionalization of carbon nanotubes, design of polymers and polymer additives, ceramics, fabrication of dendrite type materials and so on. The advantages as originally envisaged by Sharpless, the ease of fine tuning properties, less wastage, lower temperature processing and not having to remove unwanted byproducts have all been realised.

CuAAC also has been used extensively in biology and medicine. Before considering them however, the work of Bertozzi and her concept of orthogonal biology has to be described.

The standard GFP method involves attaching the gene of the green fluorescent protein to the gene of the protein being studied so that the green fluorescence of the modified protein would be a marker for monitoring protein function at cellular level. There is however, a risk of GFP interfering with the protein folding and action. More importantly, the GFP method cannot be used for elucidating the function of non protein biomolecules like Glycans. Glycans are complex chains of sugar molecules extensively found on the surface of many proteins, cells, and antibodies. They are very important in cell identification and communication.

Bertozzi was studying the role of glycans and wanted to find a good method to attach fluorescent probes to glycans. In the early 90s German scientist Reutter demonstrated that cells can be tricked into expressing the artificially modified sialic acid in their glycans. Using similar methods Bertozzi succeeded in tricking the cells to express ketones and azides in the glycan. Attempts could then be made to attach fluorescent molecules to the azides. Even before Sharpless had proposed the ideas of click chemistry Bertozzi had developed Staudinger ligation. The Staudinger reaction demonstrated by Staudinger in 1919 involved a slow two step conversion of an azide into an amine when treated with phosphine or trimethyl phosphine. Bertozzi converted this into a ligation by using an organo phosphorus compound that included fluorophores to provide the fluorescent signal. In the initial version, the complete organo phosphorus molecule was incorporated into the biological entity being investigated; a peptide or the glycan. In an improved ligation scheme, the phosphorus was deleted and only the fluorophores were left. This Staudinger ligation developed by Bertozzi has now become a very important method of bioconjugation and is extensively used. Adding fluorescent markers to glycans has been extended from living cells to live animal studies by Bertozzi and has also become a mainstream research method.

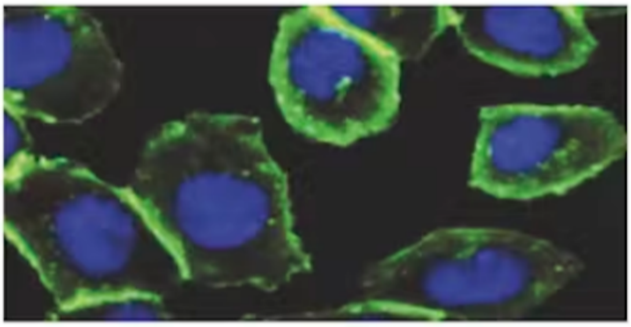


3 In the next step, Bertozzi used an alkyne that was forced into a ring-shaped molecule. The alkyne clicked with the azide.

4 A fluorescent green molecule sat on the ring-shaped molecule. This allowed Bertozzi to track the glycans on the cell's surface.

2 The modified sugar was incorporated into glycans – special carbohydrates located on the surface of cells.

1 Bertozzi fed cells with a modified sugar with an azide on it. The azide functioned as a type of molecular handle.



Bertozzi used the strain-promoted click reaction to track glycans. They have a green glow in the picture. The cell nucleus is coloured blue. Thanks to the glycans' green glow, Bertozzi was able to follow them in the cell.

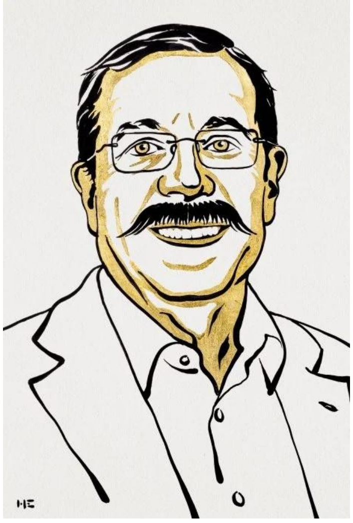
Image from *Proc Natl Acad Sci USA* [2007] 104:16793–16797

CuAAC as originally developed by Sharpless and Mendal cannot be directly used in cells or live animals because of copper toxicity. Other researchers had explored bio-friendly copper catalysts for the CuAAC click reaction using water-soluble ligands that stabilise the metal and suppress the formation of reactive oxygen species in live cells or animals. Palladium and ruthenium coupling reactions between azides and aryl iodides have also been deployed.

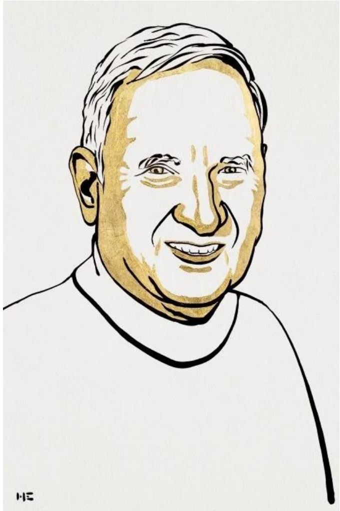
Bertozzi drew inspiration from the original work of Huisgen who showed that strained ring alkenes exhibited particularly high rates of azide alkene reaction. She demonstrated that the azide cyclooctyne reaction can has the required high reaction rate even without any catalyst. This insight was followed by other researchers who identified extremely deformed ring structures that have high reaction rates and are "bioorthogonal". This term was coined by Bertozzi and her coworkers to describe chemical reactions that can take place in the living organism without disturbing the extremely complex biochemical life processes. In the present example both azides and alkynes do not occur naturally or interfere with cell chemistry. In recent years, a tetrazine-alkene reaction has been discovered which has the highest reaction rate so far determined.

More recently, bioorthogonal bond cleaving has been developed. In this method first, the active site in a protein is caged and the protein made inactive. The cage bonds are cleaved on demand. Not only does this help in studying protein action, it becomes possible to release drugs on demand. In 2013, a team led by Marc Robillard showed trans cyclooctene(TCO)–tetrazine reaction could be used to release the anticancer drug doxorubicin on demand in living cells. In October 2020, California-based biotech company Shasqi initiated the first human use of bioorthogonal chemistry, starting a phase 1/2 clinical trial. A tetrazine-loaded hydrogel is pre injected into the tumour site, followed by infusions of the doxorubicin prodrug. Only where the two components meet, at the tumour site, is the drug unleashed, thus reducing the toxicity of the anticancer drug.

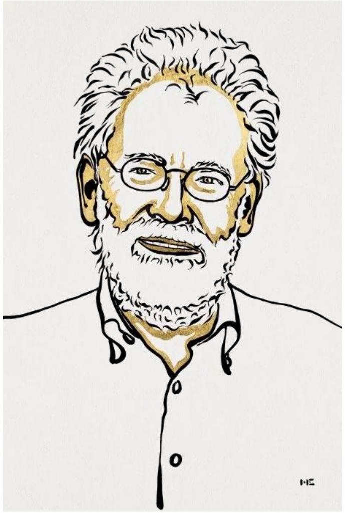
The achievements of Carolyn R. Bertozzi, Morten Meldal and K. Barry Sharpless have opened the floodgates of research activity in diverse areas. Commercial companies are competing to provide the required chemical ingredients so that the research is not confined to the elite institutions with superior facilities. It is difficult to assess the commercial use of these methods in contemporary day to day life. It is impossible to know if click chemistry has become a commercial production tool since the companies are required only to reveal product safety and not production methods. As mentioned above the first clinical trial of bioorthogonal chemistry has been initiated.



Alain Aspect,



John F. Clauser



Anton Zeilinger

The Nobel Prize in Physics 2022 was awarded jointly to Alain Aspect, John F. Clauser and Anton Zeilinger "for experiments with entangled photons, establishing the violation of Bell inequalities and pioneering quantum information science"

Each and every undergraduate course on quantum mechanics begins with a description of the experimental results that shook the foundations of classical theory of electromagnetism around the year 1900. These included the spectrum of black body radiation, Rutherford's observation of the nucleus in an atom and discrete lines in atomic spectra. Adhoc attempts at resolving these issues, also called the "old quantum theory" were made till 1927 when a unified quantum theory emerged from the work of Schroedinger and Dirac. Almost immediately, "philosophical" issues came into prominence. The then new theory implied that the quantum object has no specified properties till the measurement.

For example, consider a photon that has been created at point A at time t1 by opening a shutter and detected at time t2 at point B, using a photomultiplier tube. According to the theory, the path along which the particle moved from A to B is simply "unknowable" since it has not been experimentally determined. There is a "potential", (a vector) for each and every "possible path" between A and B. These potentials have to be vectorially added and the magnitude of the final vector provides the probability of the particle being detected at point B. Nothing more is "knowable". If there is a slit between A and B, the number of "possible paths" change and so the probability of detection at B correspondingly changes.

It is obviously silly to claim that a football kicked through a dark tunnel has no path because it is not being observed or that the planet Jupiter has no path during the day because it is not visible. Equally obviously, what is being measured in these cases is not the probability of detecting the football or the planet. Physicists "know" the situations when a quantum mechanical probability can be calculated and compared with the experiments.

Not only has quantum mechanics eliminated the problems encountered by classical electromagnetic theory, there has never been any experiment that requires a revision of quantum theory. In special cases, theory and experiment agree to the tenth decimal place which is astounding. It is equivalent to measuring and calculating the diameter of a sphere 10000 km in diameter to the accuracy of the thickness of a human hair and finding that they agree completely. So, no physicist has any alternative but to use quantum theory and in the 95 years since Schrodinger, almost everyone has given up worrying about what this theory "means". Currently all physics with the exception of the force of gravity is a quantum theory. More than a hundred years after Einstein's general theory of relativity, a quantum theory of gravity has eluded the best human efforts and many a great physicist has reconciled to this being true in the indefinite future.

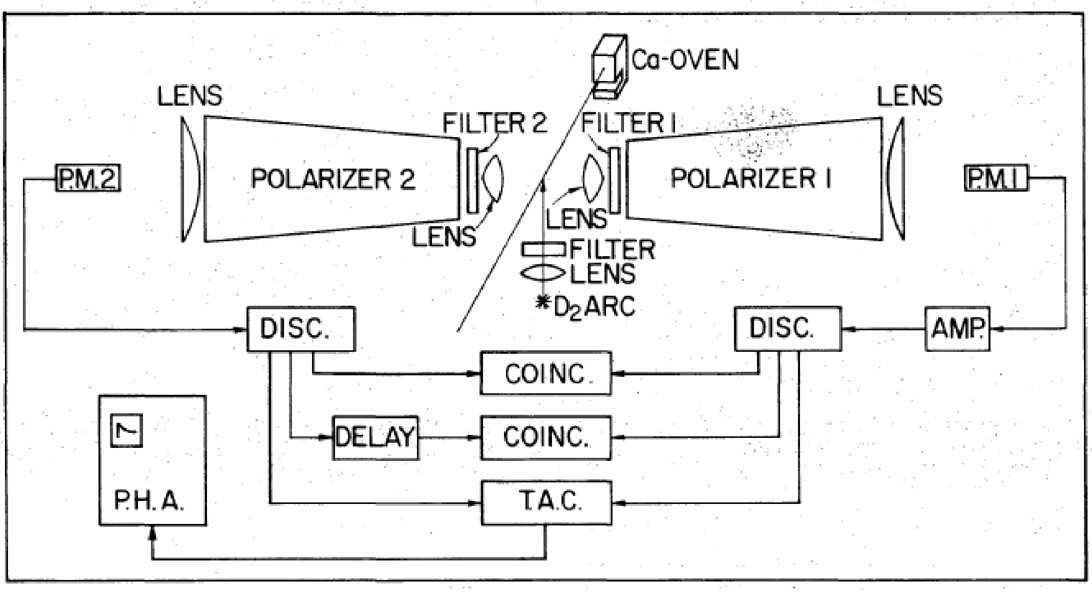
The above condensed description has to be kept in mind by anyone seeking to understand this year's award. Only then can the terms, reality, locality and entanglement be understood. Reality in this context refers to the path of the particle existing even in the absence of any experimental observation. Locality is the insistence that no signal, or communication can travel faster than "the velocity of light in vacuum". All experimentally measured quantities and properties described by the current theory of gravity are "real and local". Variables in Quantum theory obey the restriction of locality but not of "reality". Entanglement is observed when a quantum entity gets divided. This description of entangled particles is very useful. It shows that entanglement is the result of a single quantum event. A pair of entangled photons is usually produced when an atom goes from an excited state to an intermediate state releasing the first photon and then onto the ground state releasing the second. Another approach is the so called down conversion when one photon of higher energy "splits" into two photos of lower energy. The two photons together form only one quantum entity till either one is observed. The property not having a defined value till observation holds for both of them together.

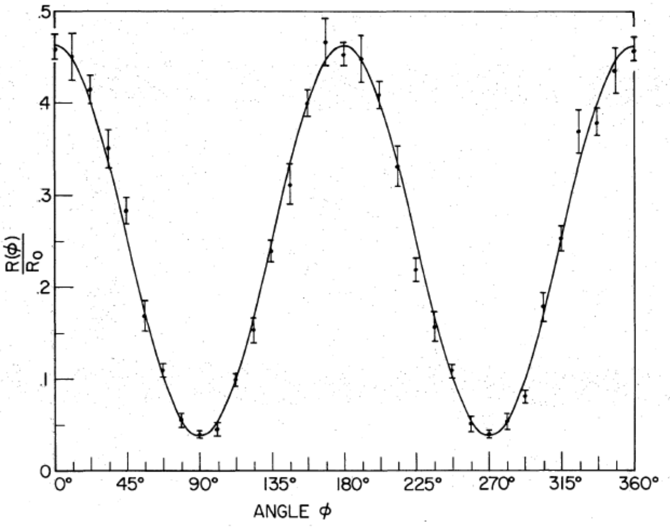
Before we discuss the actual experiments that were recognized with the award we should discuss Bell's inequality. As described above, it is impossible to "understand" even a single quantum particle, not only the entangled ones. In the single electron interference experiment, performed around the year 2000, electrons fall individually as particles. On the microscope screen they form dots. But as the dots accumulate, the interference fringes appear. No commonsensical description of how this can be is available. One is forced to accept the quantum mechanical conclusion that only the probability of the electron falling on a given location of the screen can be experimentally determined. The same inevitability of quantum explanation was demonstrated by the Stern and Gerlach experiment performed one hundred years ago. The silver atoms passing through an inhomogeneous magnetic field appear not to have a magnetic moment in a given direction before the experiment.

Long before Bell's inequalities were formulated it was apparent that a. classical theory, one which retained both reality and locality will not explain experimental observations. But this was a purely qualitative consensus. {Only Einstein continued his lonely quest for a unified field theory till he died in 1955.}

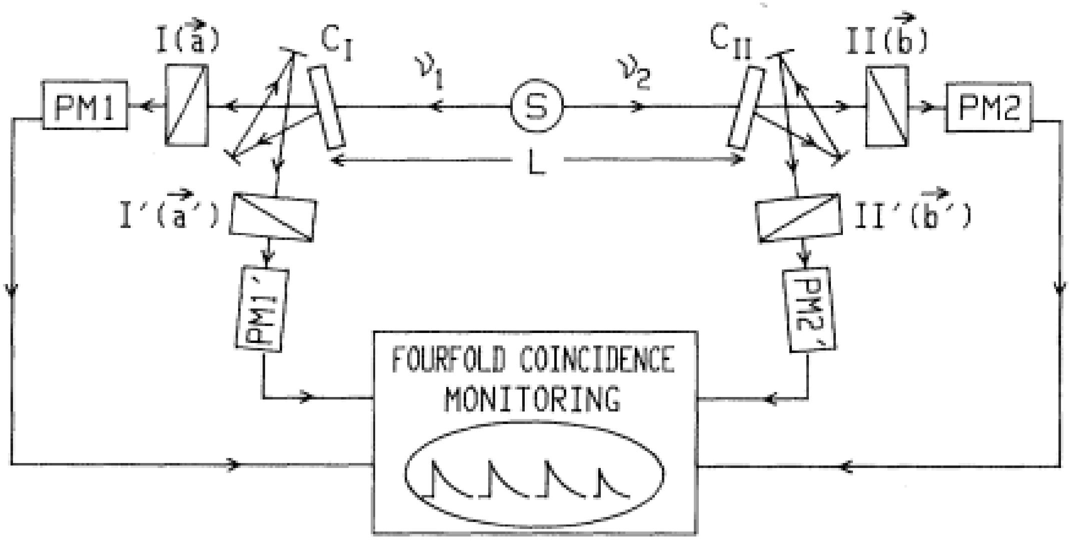
Bell's theorem converts this qualitative consensus into mathematics and gives a single number that distinguishes between any potential classical "real" theory and the "probabilistic" quantum mechanics. While experimental confirmation was several years in the future, neither Bell nor any experimenter ever had any doubt of what the result would be.

Bell considered a case of two entangled particles moving apart from each other. Experiments are performed on each particle individually to measure properties that are mutually orthogonal according to quantum mechanics. This technical language means that the two properties cannot be measured with arbitrary accuracy. It is easiest to understand this by considering the position and momentum of a particle. This is the first version of the famous Heisenberg uncertainty relation. Any attempt to measure the position more accurately will inevitably make a simultaneous measurement of momentum less accurate and vice versa. In the case of individual measurements on entangled particles, there will be a correlation between the observations. Bell's insight was that the value of correlation can be calculated both from quantum theory as also any other theory that Incorporates both "reality and locality" and further that these two values will differ. This is the famous inequality. The values of the correlation have to be calculated for each individual experimental set up.

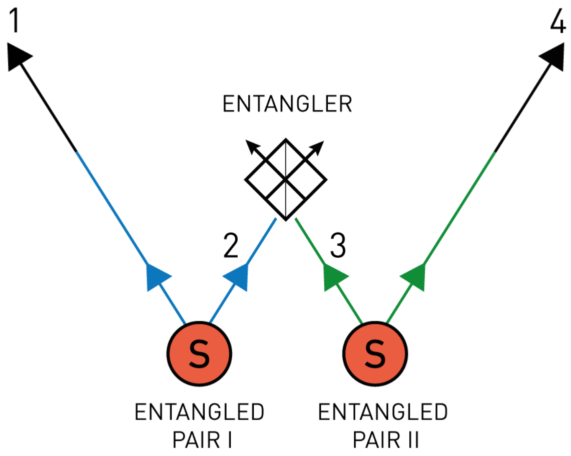


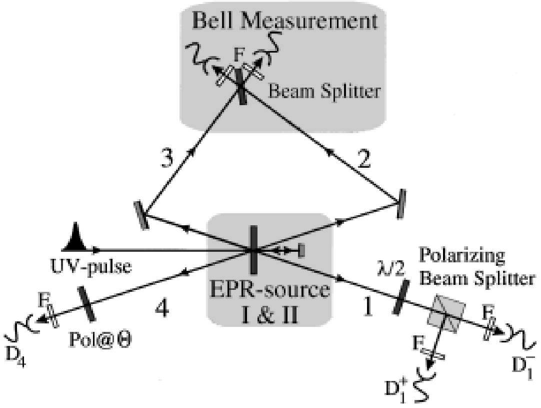


The first successful experiment (figure in previous page) to demonstrate the violation of Bell's inequality was performed by Clauser. Two correlated photons were generated and their polarisations were measured along two different directions. Simultaneous detection of photons were recorded. According to quantum theory, the probability of simultaneous detection is dependent on the angle between the two directions. Alternative theories which expect the photon to have a polarisation direction even before detection (real, local) would expect the probability to be independent of the angle. According to quantum mechanics, the probability at any angle will be higher than the probability without polarizers whereas the alternative theory would expect it to be lower. Results clearly supported quantum mechanics (figure above) It was possible to argue that the experiment was not foolproof and it was so argued. The argument was that the polaroids were not perfect and there is a possibility of signalling from one detection to another.



Aspect performed a series of progressively more efficient and sophisticated measurements to eliminate these experimental limitations that left a small loophole for these arguments in favour of the search for alternatives to standard quantum theory. In the most sophisticated version, (figure above) he employed an ultrasonic beam direction changer that directed each of the two photos into two different paths at random within 20nS, the time taken by the entangled photon to move from the source to the polarizer. The final experimental probability was 0.101+/- 0.020 close to the 0.112 calculated from quantum mechanics rather than a negative value as demanded by alternative theories. Arguments trying to find loopholes and efforts to "close the loopholes" continued to be made for over 25 years with latest experiments being reported by Zeilinger as well as other scientists even in 2017.



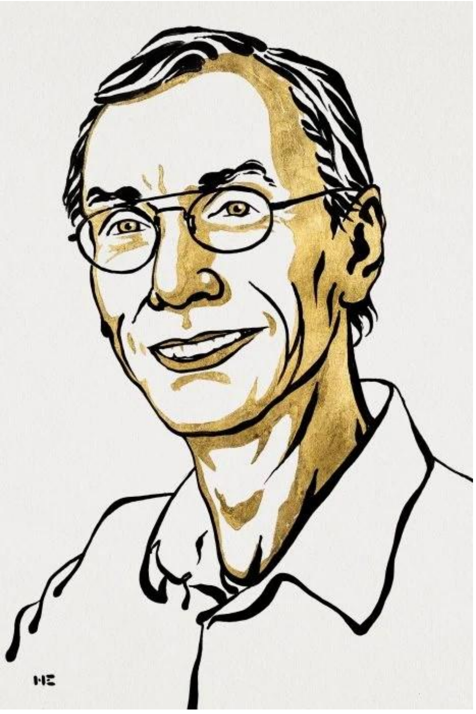


In addition to confirming the violation of Bell's inequality with more rigorous experiments, Zeilinger worked on other aspects of the entangled state. It is proved mathematically that a quantum state cannot be copied. One can intuitively understand this. The first step in copying is in some sense to "read" the quantum state and that being an experiment performed on a quantum state, the entanglement ends. Theoretically it was shown that the quantum state can be read and a copy produced provided the original is destroyed. This has been termed teleporting a quantum state since the new copy can be created at a different location. Zeilinger demonstrated this in real world systems. He performed another experiment wherein two pairs of entangled states were produced (1,2) and (3,4). Separating the pairs but getting (2,3) into an entangled state results in the pair (1,4) also getting into an entangled state (figures above).

Such results go beyond the original aim of confirming that only a probabilistic quantum theory can be in agreement with experimental results and permit the use of the entangled state in real world applications. While experiments on the counterintuitive entangled quantum entities and teleportation have been recognized by the Nobel award, they are by no means the only counterintuitive conclusions of quantum mechanics that have been translated into experiments nor are they the most weird. Other such results include the verification of the Aharonov Bohm phenomenon which is the change in quantum mechanical phase due to a completely shielded magnet, detection of Casimir force which is the force due to virtual photons, though virtual photons themselves are never detectable and quantum erasers where interference reappears when the information of the path followed by the particles is erased. The Bell inequalities appear to be more "fundamental" to those scientists reluctant to accept statistical quantum theory on non-scientific grounds. Only most people are not as honest as Einstein and admit it.

The entangled photon state is being promoted as a potential unbreakable encryption method. This would use the violation of the bell inequality as a test that there has been no eavesdropping. Any successful attempt at eavesdropping would destroy entanglement. Bell's inequality will cease to be violated and reveal the breach of security. The major problem with use of entangled photons is loss during transmission. Unlike normal optical communications, copy repeaters are not allowed. One approach to bypass this limitation is to use special space satellites. Since atmospheric losses are lower, the entangled photons can be transmitted over longer distances. Transmission of entangled states over 2400 km has been demonstrated. The other approach uses the teleportation and multiple photon tricks described above to effectively serve as repeaters. Zeiljnger has demonstrated transmission over 120 km using these methods. However, there are clearly many technical challenges to be overcome before the more substantive issues of competitive techniques, reliability and economics can even be evaluated. One important point is that entangled states are not the only way of quantum key distribution. IBM has had a working system since 1984, well before the experimental work by Zeilinger, called the BB84 which is described as a quantum key distribution but does not use engaged photons.

Painstaking and extremely difficult experimental work of Alain Aspect, John F. Clauser and Anton Zeilinger has been recognized with this year's prize in physics. However, most physicists had accepted the necessity of the probabilistic quantum theory and that too, much earlier. It is too early to assess the potential application of these experimental approaches in futuristic encryption services and other quantum technologies.

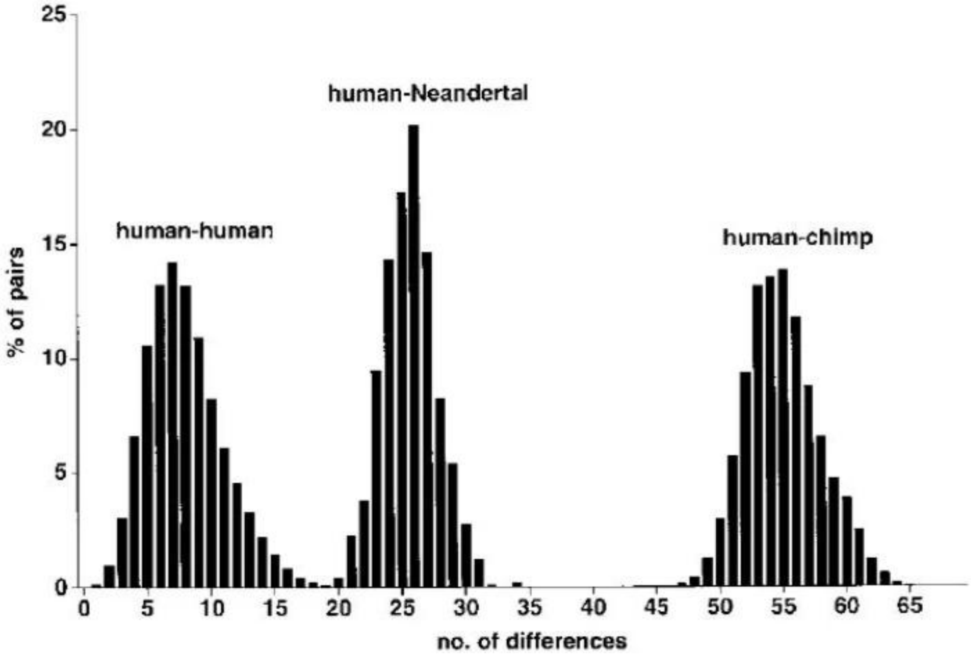


The Nobel Prize in Physiology or Medicine 2022 was awarded to Svante Pääbo "for his discoveries concerning the genomes of extinct hominins and human evolution"

Techniques of physics, chemistry and biology have been extensively used by researchers in other disciplines. Radio isotope dating is perhaps the greatest example. Geologists use the method to date rocks and historians use radiocarbon dating to know when an ancient wooden crucifix was really made. Determining the ratio of the isotopes using a mass spectrometer is the trivial part of the method. The law of radioactive decay is a law of nature and determining the half-life of an isotope is easy. The difficult part is to confirm that the sample has not been contaminated or modified in any way and that the numbers generated can be trusted. Every discipline has to identify protocols and routines to ensure thi
s.

Svante Pääbo is being recognized with this year's Nobel prize in physiology or medicine for extending the use of biological gene sequencing methods to the study of extinct huminins and thereby creating a new sub-discipline of paleogenomics. Sequencing human DNA has one advantage. There is no shortage of samples. For sequencing the DNA of extinct huminins, samples are very difficult to obtain. A couple of teeth and the piece of a single bone from a foot are considered major finds in anthropology. Further, climatic conditions at the site determine if any DNA survives in the bones. It is next to impossible for DNA in ancient bones to survive tropical or equatorial climates. Even if the bone is buried in Siberian permafrost, enhancing the chances of DNA preservation, degradation and chemical change in the nucleotides is inevitable. Even more important than all these is contamination by DNA from microbes and by modern human DNA from handling of the samples during excavation and examination. Pääbo developed a range of tests to quantify the contaminants, protocols for sample handling and tests to cross check the data. All this has more in common with a criminologist examining a few drops of blood on the crime scene than a biologist studying human physiology. Pääbo was a major if not the sole contributor to this. But this had to be completed before established modern biological techniques like Polymerase chain reaction PCR and Sanger's DNA sequencing method could be employed to investigate extinct huminin remains.

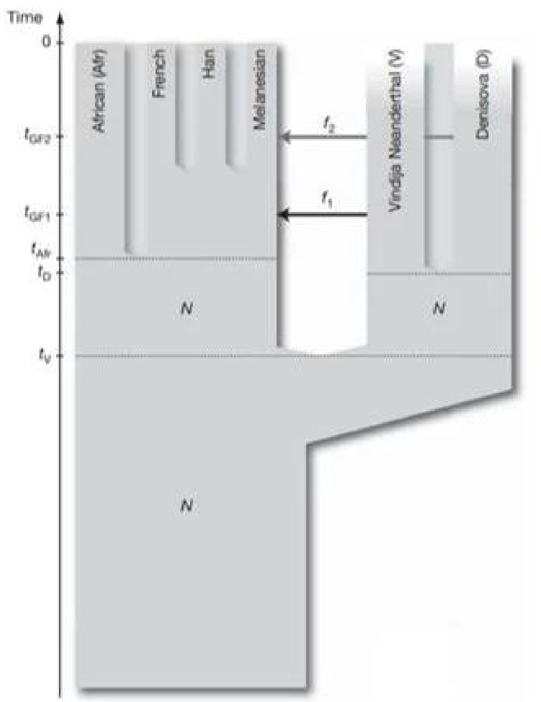
The first significant result obtained by Pääbo was on a 379 nucleotide long Neanderthal mtDNA sequence. To investigate the relationship between modern day humans and Neanderthals, the 379 nucleotides of Neanderthal mtDNA were compared with a collection of 2051 human and 59 chimpanzee mtDNA sequences.



As seen in the picture above, the number of differences between human and Neanderthal samples was on average larger than the in group differences between European, African, Asian, Native American, and Australian/Oceanic Homo sapiens. The analysis of human mtDNA which is exclusively inherited by the mothers had shown that the "mother" of all humans on earth lived around 120-150,000 years ago. This ancestor is often referred to as the mitochondrial Eve. The common matrilineal ancestor of Neanderthals and modern homosapiens is estimated to have lived around 550-690,000 years from this work. A corresponding analysis of the human Y chromosome inherited exclusively by sons from the father and called the Y chromosomal Adam is estimated to have lived around 200-300,000 years before the present.

In subsequent studies, Pääbo succeeded in sequencing the complete Neanderthal mtDNA which is 16,595 nucleotides in length. The Neanderthal mtDNA differs from that of Homo sapiens at an average of 202 nucleotide positions. Pääbo obtained another sample of bone from Denisova Cave in the Altai mountains, Russia, in a stratum dated to 48,000 to 30,000 years ago. The mtDNA he extracted from the fifth manual digit of a juvenile hominin differed at an average of 385 positions and the chimpanzee at 1,462 positions. The mtDNA from the unknown hominin was considerably more divergent from present day humans than from Neanderthal mtDNA. It was since confirmed that this was a unique hominin and named Denisova. The most recent common mtDNA ancestor shared by the Denisova hominin, Neanderthals and modern humans was estimated to have lived approximately one million years ago, or twice as old as the most recent common mtDNA ancestor of modern humans and Neanderthals. Pääbo had discovered an entirely new hominin, distinct from Neanderthals and Homo sapiens entirely by retrieval and sequencing of archaic DNA.

Pääbo began his studies of extinct hominin DNA with mtDNA because each cell has only one copy of the nuclear DNA but a very large number of mtDNA copies. But the nuclear DNA has upto a billion nucleotides whereas the mtDNA has less than 20,000 nucleotides. So he soon turned his attention to nuclear DNA. By performing a series of extremely well controlled experiments, he gradually built a sequence of the complete Neanderthal and Denisovan nuclear DNA. He could then compare these nuclear DNAs with the nuclear DNA of homosapiens from different geographical locations. He concluded that there were more Neanderthal DNA sequences in the non African homosapiens. But there was no difference between the Han Chinese, Caucasian French and Malenasian human subpopulations. There were more Denisovan DNA sequences in the Malenasians, but there were no differences between the African, Han Chinese and Caucasian French human subpopulations. This is consistent with interbreeding between the respective populations.



From the data, Pääbo concludes that the seperation of Neanderthals from homosapiens had occurred before the Denisovans had separated from the Neanderthals. It is not clarified whether Denisovans also emerged from Africa. More importantly it is inferred that the flow of genes from the Neanderthal to the homosapiens occurred before the homosapiens settled into the different geographical regions, namely europe, china and the islands but that the flow of genes from the Denisovans had occurred later. Shown as f1 and f2 in the picture.

While celebrating this result, obtained by Pääbo with the help of a large number of painstaking and precise experiments, one should not overlook how obvious the result actually is. Since the time of homo erectus, human lifestyle has been of a hunter-gatherer with stone tools and fire. All morphological evidence suggests that the differences between Neanderthals and homosapiens are minor and would not have been obvious to untrained observers. Is interbreeding among such groups sharing the same area and same lifestyle such a surprise? Particularly when female ovulation in humans is concealed? Would not disappearance of the specific morphology of Neanderthals be more easily understood as the consequence of interbreeding with homosapiens, a group with higher fecundity? Particularly since the Neanderthal population is found to be very small? What is the explanation for interbreeding not altering the mtDNA? Should not proof of the presence of the Denisovan in high altitude locations for an extended period of time be a prerequisite before claims that interbreeding conferred advantages on the incoming homosapiens are entertained?

All the sophistication in the experimental techniques will not eliminate the inherent uncertainties in anthropology which will remain a social science. That is the lesson from radiocarbon dating as was mentioned right at the start. Uncertainty is not a big issue in social science. However, when the work is selected for the highest award in the area of physiology and medicine and attempts are made to justify the award by citing weak social science claims as potential benefits, the discrepancy becomes too glaring to be glossed over.

To be sure while science itself may be objective, selecting a work for recognition and reward is not. Omissions and commissions can always be identified. Over the past fifteen years while making such presentations I have commented occasionally on such award s. Nevertheless, my usual conclusion that Scientific understanding creates happiness even in absence of creativity stands. One can excuse one’s own lack of personal contribution by noting that the creator needs an appreciative audience and hopefully the present attempt does succeed in creating in the audience an appreciation of the Nobel Prizes in Science for 2022.